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Center for Veterinary Biologics
Testing Protocol

SAM 509

Supplemental Assay Method for the Mikrozone Electrophoretic Separation of Blood Fractions Used in Veterinary Biologics

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1. Introduction

This Supplemental Assay Method (SAM) describes the procedure used to determine the percent albumin or globulin present in a serum, antiserum or antitoxin. Combined with an assay for total protein, it can determine the protein content of the serum.

2. Materials

Brand names are provided for reference only. Equivalent equipment or materials may be used.

2.1 Equipment/instrumentation

2.1.1 Electrophoresis equipment (microzone cell) with power supply--(Brinkman Sartorius Microphor)

2.1.2 Microzone densitometer

2.1.3 Cellulose acetate strips--Size 57 x 145-mm (Sartorius cellulose acetate for electrophoresis Catalog No. 11207)

2.1.4 Microzone sample applicator--(Beckman Catalog No. 324399)

2.1.5 Computer--(with Densitometer integration calculation program)

2.1.6 Common laboratory apparatus and glassware-- beakers, broad-tipped forceps (tweezer style), white paper towels or white filter paper, pen with non-diffusing ink, stainless steel pans with lids (8.5” x 5” x 2”), ruler, class A volumetric flasks, parafilm, pipets or pipettor with tips

2.2 Reagents/supplies

All chemicals are reagent grade unless specified. Store at room temperature unless otherwise specified. Chemicals and solutions are good for 1 year unless otherwise specified.

2.2.1 Tris-barbital-sodium barbital buffer--(Gelman High Resolution Buffer Cat# 51104), pH 8.8. Undiluted buffer stable for 2 years.

2.2.2 Ponceau S fixative dye solution--(Beckman Cat# 324340) Store stock at 4°C. Stock stable for 2 years.

2.2.3 Acetic Acid--(Glacial--greater than 95%)

2.2.4 Bovine serum reference--normal bovine serum [NVSL media# 40032, bovine serum (donor)]. Store frozen (-20° C).

2.2.5 Water--use deionized or distilled water, or water of equivalent purity.

3. Preparation for the test

3.1 Personnel qualifications/training

No specific training is required. Individuals should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Preparation of equipment/instrumentation

3.2.1 Prepare electrophoresis cell

3.2.1.1 Place cell on horizontal surface and fill the buffer compartments to the fill line with tris-barbital-sodium barbital pH 8.8 buffer solution (**3.3.1**).

3.2.1.2 With laboratory toweling, wipe any buffer from the edges and center section of the cell.

3.2.2 Prepare cellulose acetate strips

Critical control point: Touch the cellulose acetate strip as little as possible, use tweezers.

3.2.2.1 Using a pen with non-diffusing ink, mark each strip with a reference line 2 inches from the left end of the strip. Write all sample numbers, dates and other information on the left end of the strip.

3.2.2.2 Gently float the strip on the surface of buffer solution which has been poured into a flat stainless steel pan. When the strip is wetted, use tweezers to gently submerge the strip in the buffer.

Critical control point: Do not trap air bubbles between the strip and the buffer solution. Be careful to not submerge the strip before it is thoroughly wetted.

3.2.2.3 After approximately 5 minutes, carefully remove strip from the buffer and remove excess buffer by gently blotting it between two sheets of white toweling or two filter paper sheets.

Critical control point: Blot only until excess moisture is removed--Do not over dry (dry spots will appear on the strip).

3.2.2.4 Carefully stretch the blotted cellulose acetate strip across the bracket and place in the electrophoresis cell with the ends of the strip hanging directly in the buffer.

3.3 Preparation of reagents/control procedures

3.3.1 Tris-barbital-sodium barbital buffer: Dilute contents of one packet with water following label directions. Mix until completely dissolved. Buffer stable for 6 to 8 weeks if stored at 4°C, warm to room temperature before using.

3.3.2 Ponceau S working solution: Dilute contents of one container of stock dye with water following label directions.

3.3.3 Acetic acid, 5%: Carefully add 50 ml glacial acetic acid to 600 ml water in 1000-ml volumetric flask. Mix and fill to volume with water.

3.3.4 Serum reference: Thaw serum, warm to room temperature and mix gently by inverting vial 6-8 times.

3.4 Preparation of sample

3.4.1 Serum products (blood origin products) and serum samples are usually ready for electrophoresis. Serum fractions produced by ammonium sulfate or sodium sulfate precipitation must be dialyzed to remove high salt content. See current version of **TCSOP0001** for receipt of sample.

3.4.1.1 Using a pipet or pipettor with tip, place a drop of sample (gently mixed) on a sheet of parafilm with a sample number marked to identify each drop. (Include reference serum with each run.)

3.4.1.2 Use a microzone sample applicator to apply the samples to the strip, with the application points near the reference line (approximately 1/2-inch to the right of the line). Be careful that only the tip of the applicator touches the sample droplet and that the applicator tip is rinsed and blotted dry between sample applications. Run duplicates of each sample and the reference serum. (Run duplicates of the reference on <u>each</u> strip.)

4. Performance of the test

4.1 Electrophoresis run

4.1.1 Place cover on the electrophoresis cell and, attach electrical connections to the power supply. Run current from "left" to "right" end of strip.

4.1.2 Set current to constant voltage and switch power on. Adjust voltage to 250 volts.

4.1.3 Run migration for 25 minutes.

4.1.4 Turn off power and disconnect cell from the power supply. The same batch of buffer may be used for more than one run but the current must be reversed with each additional run (changing the sides of the anode and the cathode on the cell). The strips must also be reversed, so migration is in the same direction on the strip.

4.2 Staining

4.2.1 Carefully remove the strip from the electrophoresis cell. Float strip on staining solution, as with buffer (**Section 3.2.2.2**), and then submerge. Allow to stain for approximately 5 minutes.

Critical control point: Do not place one strip over another in the staining solution, stain strips individually.

4.2.2 Remove strip from the stain and rinse in 3 rinses of 5% acetic acid--approximately 5 minutes in each rinse. If the background is not white, repeat the rinses.

4.2.3 Remove the strip from the last rinse and blot with clean white toweling. Check to determine if the protein fractions appear as distinct red bands. If bands appear in proper places and stain well, place the strip flat between sheets of toweling and press with a heavy, broad-surfaced object until dry.

4.2.4 If reference sample does not migrate or stain properly, repeat run.

5. Report of test results

5.1 Integrate patterns with the densitometer. Use the ink mark on the strip as an end point reference. Determine the albumin and globulin percentages using the area of the peaks in the integrated pattern.

5.2 Determine the albumin (A) or globulin (G) fraction (%) by dividing the A (or G) integrator area by the total integrator area. To determine the A (or G) concentration (mg/ml), multiply that fraction by the total protein value of the sample (determine total protein concentration according to current version of **TCSAM0504**). A computer program can be used for these calculations. The A/G ratio is normally calculated for each sample.

6. Report of test results

Test results are reported following the current version **TCSOP0001.**

7. References

7.1 Reference is made to the long time use of this procedure by the Toxicology/Chemistry Section.

8. Summary of revisions

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the name of the contact person has changed.